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INHIBITORY EFFECT OF HISTAMINE ON THE RELEASE OF MELANIN-DISPERSING HORMONE IN THE FIDDLER CRAB, *UCA PUGILATOR*

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Abstract

Histamine (HA), a stimulator of H_1 and H_2 receptors, produced dose-dependent inhibition of the melanin dispersion which normally occurs when fiddler crabs, *Uca pugilator*, are transferred from a white to a black background. The HA precursor L-histidine, and 4-methyl histamine (4-MeHA), an H_2 receptor agonist, also inhibited melanin dispersion. 2-Methyl histamine (2-MeHA), an H_1 receptor agonist, enhanced melanin dispersion. The inhibitory effects of HA and 4-MeHA were abolished by the H_2 receptor blocker metiamide but not by blockers of either H_1 receptors or $alpha_1$ adrenoceptors. Melanin-dispersing hormone (MDH) release is accomplished mainly by stimulation of $alpha_1$ adrenoceptors with norepinephrine appearing to be the neurotransmitter involved. The H_1 receptor blockers pyrilamine and SA-97 antagonized 2-MeHA. HA-induced inhibition of melanin dispersion was potentiated by the noradrenergic neuron blocker bretylium and the $alpha_2$ adrenoceptor agonist B-HT 933. HA did not significantly affect melanin dispersion in crabs pretreated with 6-hydroxydopamine which destroys catecholaminergic neuroterminals. None of these drugs affected the melanophores directly. On the basis of these and previously obtained results it is suggested that H_1 and H_2 receptors are present on norepinephrine neurons involved in triggering MDH secretion, and administered HA inhibits MDH release by decreasing impulse-mediated noradrenergic neurotransmission through stimulation of H_2 receptors.

Introduction

Migration of the melanin in the melanophores of the fiddler crab, *Uca pugilator*, is controlled by antagonistic hormones, a melanin-dispersing hormone (MDH) and a melanin-concentrating hormone (Carlson, 1935; Sandeen, 1950; Fingerman, 1956). Release of MDH in this fiddler crab appears to be triggered by noradrenergic neurotransmission (Hanumante *et al.,* 1980, 1981; Fingerman *et al.,* 1981; Hanumante and Fingerman, 1981; 1982a, b, c).

In mammals, at least, injected histamine (HA) inhibits impulse-mediated norepinephrine (NE) release, but whether HA has a physiological role in local regulation of neurotransmission has not yet been completely established (Westfall, 1980). HA has also been suggested to have a neurotransmittory role in stimulating the release of a corticotropin-releasing hormone (Allolio *et al.*, 1981) and a prolactin (Donoso and Banzan, 1980) in mammals. With respect to crustaceans, HA has until now only been reported to be present in the nervous systems of the crabs *Carcinus maenas* (Kerkut and Price, 1961; Clay, 1968; Huggins and Woodruff,

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Abbreviations: HA, histamine; MDH, melanin-dispersing hormone; 2-MeHA, 2-methyl histamine; 4-MeHA, 4-methyl histamine; NE, norepinephrine; 6-OHDA, 6-hydroxydopamine.

1968; Woodruff *et al.*, 1969) and *Cancer borealis* (Clay, 1968). However, the role of HA, if any, in the physiology of crustaceans remains unknown. Therefore, it was considered important from the point of comparative pharmacology to determine whether HA has any direct or indirect effect on the release of MDH in the fiddler crab, *Uca pugilator*. This aim was implemented by administering highly specific histaminergic agents and noting their effects on the degree of pigment dispersion in the melanophores of crabs kept either on a white or a black background throughout the experiment or of crabs transferred from a white to a black background or from a black to a white background at the time the drugs were injected.

Materials and Methods

Adult male fiddler crabs, *Uca pugilator*, from the area of Panacea, Florida (Gulf Specimen Co.) were used. Their melanophores were staged using the system of Hogben and Slome (1931). According to their scheme, stage 1.0 represents maximal pigment concentration, stage 5.0 maximal dispersion, and stages 2.0, 3.0, and 4.0 the intermediate conditions. To facilitate comparison of responses of the experimental and control crabs, the mean responses of both groups were used to calculate "Response Indices" as defined by Hanumante and Fingerman (1982a). The Response Index is the difference between the means of the control and experimental groups. For example, if the mean melanophore stage of the control group was 2.8 and that of the experimental group 3.6, then the Response Index is +0.8, the "+" signifying the melanin of the experimental group was more dispersed than that of the control group. A "−" signifies that the melanin of the experimental group was less dispersed than that of the control group. Each value presented below is based on average melanophore stage of 10 intact experimental crabs or 10 experimental isolated legs. The same number of control crabs and isolated legs was used. The data were analyzed by means of Student's *t* test with significance set at the 95% confidence interval. Standard Errors of the Means were also calculated.

When assays were performed on intact crabs, their melanophores were staged at the time of injection and 15, 30, 60, 90 and 120 minutes thereafter. However, when assays were performed on isolated legs, the melanophores were staged only at the time the legs were removed from the crab (at which time the legs were perfused with the test or control solution) and 15, 30, 45, and 60 minutes thereafter. The melanophores in isolated legs of this crab remain responsive for at least 120 minutes (Herman and Dallmann, 1975). However, in order to make sure that the melanophores we used in our *in vitro* experiments would indeed also be viable, isolated legs were challenged with eyestalk extract (containing 1/3 eyestalk equivalent/leg), the eyestalk being the major source of MDH in *Uca pugilator* (Sandeen, 1950). The volume of solution injected into each intact crab was always 0.05 ml. Likewise, each isolated leg was perfused with 0.05 ml of the appropriate solution. When intact crabs were used, the melanophores seen through the cuticle on the anteroventral surface of the second walking leg on the right side were staged. When isolated legs were used, the second and third walking legs from both sides of the crabs were removed, the legs from the left side serving as controls for the legs on the right side that received the test solution, and the melanophores on the anteroventral surface of these isolated legs were likewise observed for staging. These experiments with intact crabs and isolated legs were performed at 24°C under an illumination of 1190 lx.

Six of the drugs used in the present investigation, namely, metiamide (N-

methyl-N′ {2 [5-methylimidazol-4-yl) methylthio]ethyl} thiourea) (Smith, Kline and French), 2- and 4-methyl histamine dihydrochloride (Smith, Kline and French), bretylium tosylate (American Critical Care), SA-97 (homochlorocyclizine) (Eisai), and B-HT 933 (2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo-{5,4-d}-azepin dihydrochloride) (Boehringer Ingelheim) were generous gifts. In addition, rauwolscine hydrochloride (Roth), coryanthine hydrochloride (Sigma), HA (Sigma), pyrilamine maleate (Sigma), 6-hydroxydopamine hydrobromide (Sigma), and L-histidine monohydrochloride (Sigma) were used. The concentration used for each drug was always 20 μg/dose of the free compound in assays on both whole animals and isolated legs except in two experiments, where the responses to different doses of HA were determined and when 15 μg HA were combined with 20 μg metiamide. All the drugs were dissolved in Pantin's physiological saline (Pantin, 1934). To dissolve the metiamide, a drop of HCl (1.2 *M*) was added to the saline. Consequently, a drop of HCl (1.2 *M*) was also added to the control saline for the metiamide experiments. The rest of the controls received pure saline.

Experiments and Results

Effects of histaminergic agents on melanin migration

The aim of this series of experiments was to determine whether HA and other agents acting through histaminergic mechanisms affect melanin migration in the fiddler crab. Mammals have two types of HA receptors, called H_1 and H_2 (Douglas, 1980; Schwartz *et al.*, 1980; Polanin and McNeill, 1981). The two classes of HA receptors were revealed by the differential responses of experimental animals to histaminergic agonists and antagonists. Histidine is a precursor of histamine (Douglas, 1980). 2-Methyl histamine (2-MeHA) is a specific agonist of H_1 receptors (Ash and Schild, 1966; Douglas, 1980), whereas 4-methyl histamine (4-MeHA) selectively stimulates H_2 receptors (Black *et al.*, 1972; Durant *et al.*, 1975; Owen *et al.*, 1979; Douglas, 1980; Polanin *et al.*, 1981; Tenner, 1981). None of these drugs produced melanin dispersion in crabs initially having maximally concentrated pigment and which continued to be kept on a white background throughout the experiment (Table I). However, a preliminary experiment had revealed that HA inhibited melanin dispersion in crabs transferred from a white to a black background. The melanin of fiddler crabs disperses in specimens transferred from a white to a black background and concentrates following transfer from a black to a white background. An experiment was then performed to determine whether this effect is dose related. For this purpose, four HA concentrations, namely 5, 10, 15, and 20 μg/dose, were administered to crabs having maximally concentrated melanin initially. These crabs together with controls underwent a background change from white to black immediately after receiving the HA or saline. It is evident from Figure 1 that HA produced dose-dependent inhibition of melanin dispersion.

L-Histidine and 4-MeHA, like HA, also significantly decreased the rate of melanin dispersion in crabs initially having fully concentrated melanin and which were shifted from a white to a black background (Table II). In contrast, 2-MeHA significantly enhanced the rate of melanin dispersion in crabs that underwent a background change from white to black and whose melanin was initially maximally concentrated (Table II).

In crabs, initially having maximally dispersed melanin and which were kept in black pans throughout the experiments, HA, L-histidine, and 4-MeHA produced significant inhibition in melanin dispersion (Table I), but 2-MeHA kept the melanin from concentrating to the extent evident in the saline-injected control crabs. The

TABLE I

Response Indices of melanophores of crabs administered a drug and maintained throughout the experiment on a white (W) or a black (B) background.

Drug	Background	Time (minutes) 15	30	60	90	120
Histamine	W	−0.1	0.0	0.0	0.0	−0.1
	B	−0.8*	−0.8*	−0.7*	−0.9*	−0.7
L-Histidine	W	0.0	0.0	0.0	0.0	0.0
	B	−0.2	−0.6	−0.3	−0.3	−0.8*
2-Methyl histamine	W	0.0	0.0	0.0	0.0	0.0
	B	+0.4	+0.5	+0.6*	+0.6*	+0.4*
4-Methyl histamine	W	0.0	0.0	0.0	0.0	0.0
	B	−0.2*	−0.2*	−0.4*	−0.4*	−0.4*

+, melanin more dispersed in experimentals than in controls; −, melanin less dispersed in experimentals than in controls.

At the outset (0 minute time), response indices for all groups of crabs were 0.0.

* Statistically significant, $P \leqq 0.05$ as compared with controls.

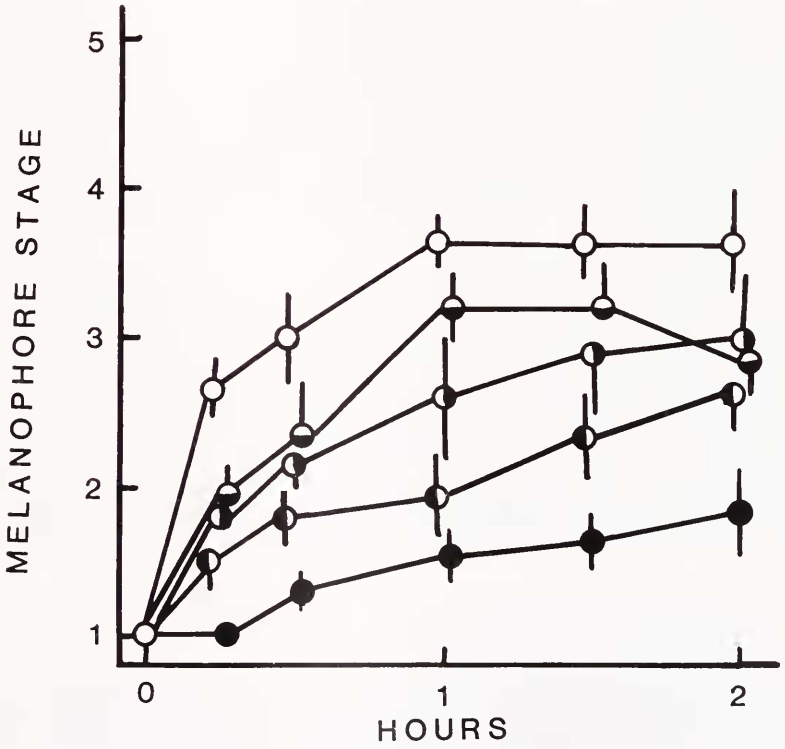

FIGURE 1. Relationships between melanophore stage and time for fiddler crabs, *Uca pugilator*, transferred from a white to a black background. Solid circles, crabs that received 20 μg histamine; circles half-filled on left, crabs that received 15 μg histamine; circles half-filled on right, crabs that received 10 μg histamine; circles half-filled on bottom, crabs that received 5 μg histamine; open circles, saline-injected controls. Vertical bars indicate SEM.

TABLE II

Response Indices of melanophores of crabs administered 20 μg of a drug and then changed from a white to a black background (W → B) or from a black to a white background (B → W).

Drug	Background	Time (minutes)				
		15	30	60	90	120
Histamine	W → B	−1.7*	−1.7*	−2.1*	−2.0*	−1.8*
	B → W	−0.3	−0.4	−0.1	−0.2	−0.5
L-Histidine	W → B	−1.2*	−1.1*	−1.4*	−1.0*	−1.0*
	B → W	+0.2	−0.1	−0.3	−0.3	−0.2
2-Methyl histamine	W → B	+0.1	+0.3	+0.6*	+0.3	+1.0*
	B → W	+0.2	+0.6*	+0.5	+0.6	+0.6*
4-Methyl histamine	W → B	+0.2	−0.7*	−1.1*	−1.2*	−1.6*
	B → W	−0.3	−0.5*	−0.6*	−0.3	−0.3

+, melanin more dispersed in experimentals than in controls; −, melanin less dispersed in experimentals than in controls.

At the outset (0 minute time), response indices for all groups of crabs were 0.0.

* Statistically significant $P \leqq 0.05$ as compared with controls.

melanin concentration observed in the control crabs maintained on a black background and whose melanin was initially in stage 5.0, was probably a result of excitement blanching caused by handling of the crabs when readings were taken and/or the circadian rhythm of color change (which fosters dispersion of the pigment during the daytime and concentration of the pigment at night), and/or the circatidal rhythm of color change which modulates this circadian rhythm.

HA and L-histidine did not significantly affect the rate of melanin concentration in crabs shifted from a black to a white background and whose melanin was initially maximally dispersed (Table II). However, 2-MeHA significantly slowed and 4-MeHA significantly increased the rate of melanin concentration in the crabs initially having fully dispersed melanin and which were shifted from a black to a white background. None of these histaminergic agents significantly affected pigment migration in the melanophores of isolated legs (Table III). On the contrary, MDH (eyestalk extract) was effective in keeping melanophores in isolated legs significantly more dispersed over saline-perfused legs (Table III), thereby confirming that our *in vitro* system was viable. Regardless of whether the melanin is maximally concentrated or maximally dispersed at the time a leg is removed, this pigment tends to attain an approximately intermediate degree of dispersion in isolated legs.

Effects of H_1 and H_2 receptor antagonists on melanophore responses to histamine and histamine agonists

The objective of this series of experiments was to characterize the receptors which mediate the HA-induced inhibition in melanin dispersion. Pyrilamine and SA-97 are specific H_1 receptor antagonists (Ash and Schild, 1966; Donoso and Banzan, 1980; Douglas, 1980; Gotow *et al.*, 1980), whereas metiamide selectively blocks H_2 receptors (Black *et al.*, 1972; Donoso and Banzan, 1980; Douglas, 1980). Crabs having maximally concentrated melanin initially and which underwent a

TABLE III

Response Indices of melanophores in isolated legs administered a drug or an eyestalk extract (½ eyestalk/crab).

Drug	Initial state of pigment	Time (minutes) 15	30	45	60
Histamine	MC	+0.1	+0.2	+0.1	0.0
	MD	0.0	0.0	0.0	−0.2
L-Histidine	MC	0.0	+0.3	+0.4	−0.2
	MD	−0.2	+0.1	+0.3	+0.2
2-Methyl histamine	MC	−0.4	+0.1	+0.1	−0.2
	MD	+0.1	+0.3	+0.3	+0.1
4-Methyl histamine	MC	−0.2	+0.1	+0.2	+0.2
	MD	0.0	+0.1	+0.1	0.0
Pyrilamine	MC	+0.2	+0.1	−0.1	−0.1
	MD	+0.2	−0.1	0.0	0.0
SA-97	MC	+0.2	+0.3	+0.2	+0.2
	MD	+0.1	+0.1	0.0	0.0
Metiamide	MC	−0.1	0.0	−0.4	−0.1
	MD	+0.1	0.0	−0.2	−0.1
Eyestalk extract	MC	+0.7*	+0.7*	+0.5	+0.8*
	MD	+0.5*	+1.2*	+1.4*	+1.7*

+, melanin more dispersed in experimentals than in controls; −, melanin less dispersed in experimentals than in controls.

At the outset (0 minute time), response indices for all groups of crabs were 0.0.

MC = Maximally concentrated, MD = Maximally dispersed.

* Statistically significant $P \leqq 0.05$, as compared with controls.

background change from white to black at the time of drug injection were used in this set of assays.

None of the H_1 and H_2 receptor blockers by themselves significantly affected melanin migration *in vivo* (Fig. 2A) or *in vitro* (Table III). Furthermore, the H_1 receptor blockers did not significantly antagonize the HA-produced decrement in the rate of melanin dispersion (Fig. 2B), but significantly decreased the 2-MeHA-induced increase in melanin dispersion (Fig. 3A). Of the two H_1 receptor blockers, SA-97 was more effective. Metiamide significantly overcame the HA and 4-MeHA-induced melanin concentration (Figs. 3B and 4A). However, the inhibitory effect of metiamide against HA while significant during the first 30 minutes of the experiment was not apparent after one hour.

Effects of noradrenergic agents on the histamine effects on melanophores

This series of experiments was devised to determine whether the HA-induced inhibition of melanin dispersion is due to interaction of HA with a noradrenergic mechanism. Several drugs were used. Coryanthine preferentially blocks alpha$_1$ adrenoceptors (Tanaka and Starke, 1980; Weitzell *et al.*, 1979; Timmermans *et al.*, 1981). Rauwolscine selectively blocks alpha$_2$ adrenoceptors (Tanaka and Starke, 1980; Weitzell *et al.*, 1979; Timmermans *et al.*, 1981) whereas B-HT 933 is a

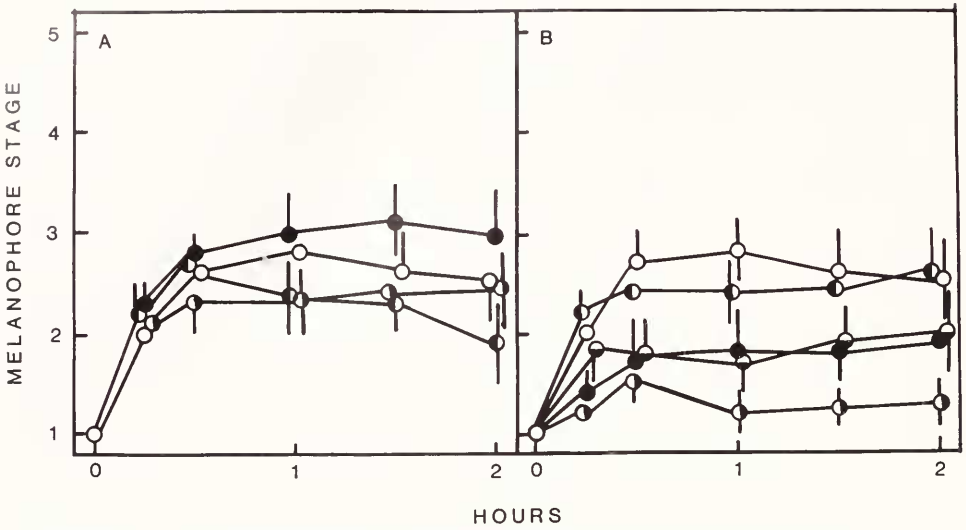

FIGURE 2. Relationships between melanophore stage and time for fiddler crabs, *Uca pugilator*, transferred from a white to a black background. (A): Solid circles, crabs that received metiamide; circles half-filled on left, crabs that received pyrilamine; circles half-filled on right, crabs that received SA-97; open circles, saline-injected controls. (B): Solid circles, crabs that received histamine; circles half-filled on left, crabs that received SA-97; circles half-filled on right, crabs that received histamine plus SA-97; circles half-filled on bottom, crabs that received histamine plus pyrilamine; open circles, saline-injected controls. Vertical bars indicate SEM.

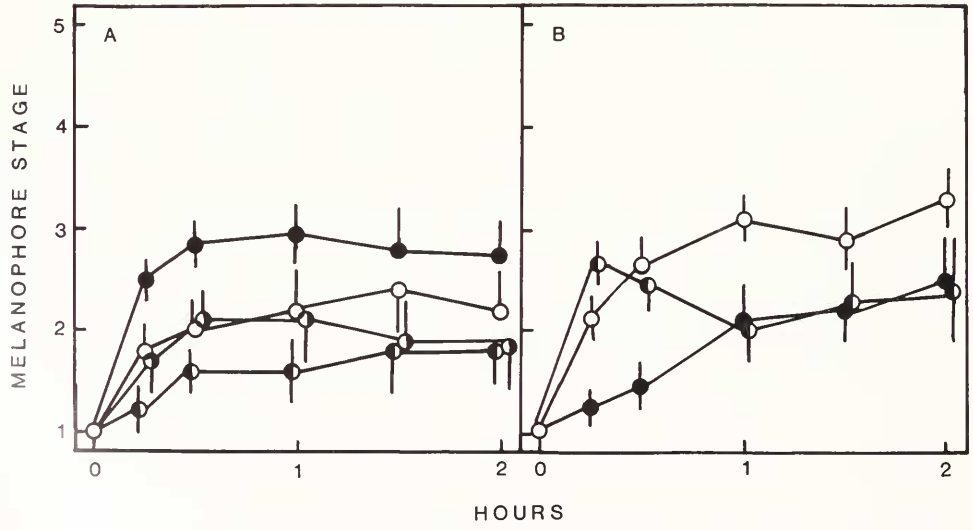

FIGURE 3. Relationships between melanophore stage and time for fiddler crabs, *Uca pugilator*, transferred from a white to a black background. (A): Solid circles, crabs that received 2-methyl histamine; circles half-filled on left, crabs that received 2-methyl histamine plus SA-97; circles half-filled on right, crabs that received 2-methyl histamine plus pyrilamine; open circles, saline-injected controls. (B): Solid circles, crabs that received histamine; circles half-filled on left, crabs that received histamine plus metiamide; open circles saline-injected controls. Vertical bars indicate SEM.

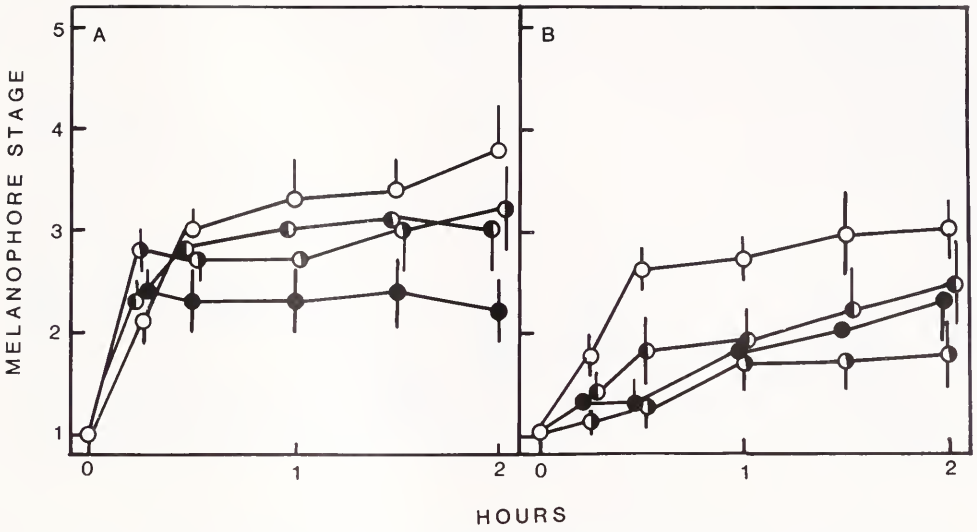

FIGURE 4. Relationships between melanophore stage and time for fiddler crabs, *Uca pugilator*, transferred from a white to a black background. (A): Solid circles, crabs that received 4-methyl histamine; circles half-filled on left, crabs that received metiamide; circles half-filled on right, crabs that received 4-methyl histamine plus metiamide; open circles, saline-injected controls. (B): Solid circles, crabs that received histamine; circles half-filled on left, crabs that received coryanthine; circles half-filled on right, crabs that received histamine plus coryanthine; open circles, saline-injected controls. Vertical bars indicate SEM.

highly selective alpha$_2$ adrenoceptor agonist (Kobinger, 1976; Hammer *et al.*, 1980; Timmermans and van Zwieten, 1980a, b; Pichler and Kobinger, 1981). Bretylium is a noradrenergic neuron blocker which specifically prevents impulse-mediated release of NE from presynaptic neurons (Boura and Green, 1959; Natoff and Dodge, 1980) whereas 6-hydroxydopamine (6-OHDA) selectively destroys catecholaminergic neuroterminals, including those of NE (Kostrzewa and Jacobowitz, 1974; Hwang *et al.*, 1980; Ritzmann and Bhargava, 1980). Also in this series of experiments crabs which were shifted from a white to a black background at the time of injection and whose melanin was maximally concentrated initially were used.

Coryanthine, by itself, like HA, significantly reduced the rate of melanin dispersion (Fig. 4B). The combination of HA and coryanthine also significantly decreased the rate of centrifugal melanin migration from that of the control crabs, but this decrement was not significantly less than that observed in the crabs that received either drug alone.

In order to obtain further support for the concept that HA-induced inhibition in melanin dispersion is mediated through H_2 receptors, the effects of 4-MeHA and coryanthine were determined. This combination reduced the rate of melanin dispersion which differed significantly from that of the control crabs as well as of the crabs that received either 4-MeHA or coryanthine. Each drug alone significantly reduced the rate of centrifugal melanin migration (Fig. 5A).

B-HT 933 (Fig. 5B) and bretylium (Fig. 6A) by themselves, like HA, kept the melanin less dispersed than in the respective control crabs. The combinations of HA and B-HT 933 (Fig. 5B) and HA and bretylium (Fig. 6A), further significantly

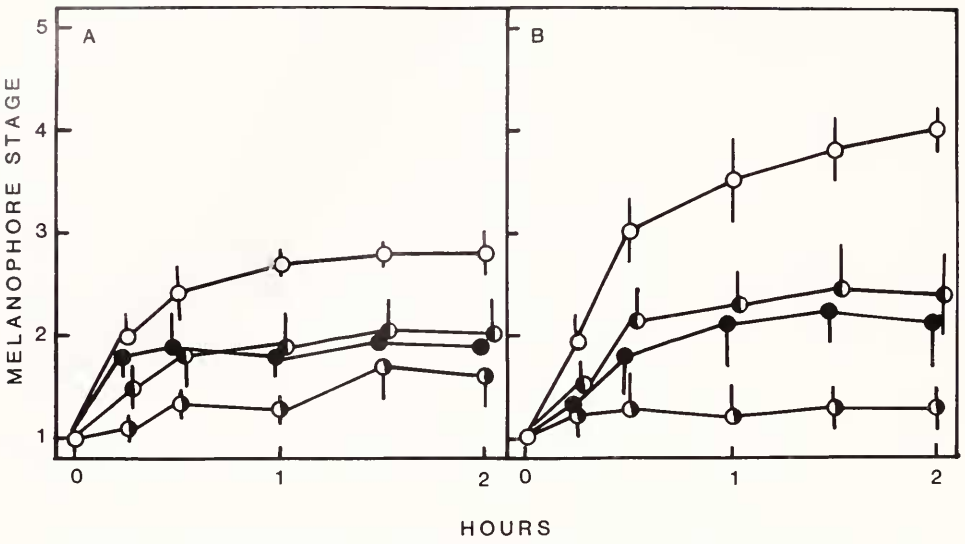

FIGURE 5. Relationships between melanophore stage and time for fiddler crabs, *Uca pugilator*, transferred from a white to a black background. (A): Solid circles, crabs that received 4-methyl histamine; circles half-filled on left, crabs that received coryanthine; circles half-filled on right, crabs that received 4-methyl histamine plus coryanthine; open circles, saline-injected crabs. (B): Solid circles, crabs that received histamine; circles half-filled on left, crabs that received B-HT 933; circles half-filled on right, crabs that received histamine plus B-HT 933; open circles, saline-injected controls. Vertical bars indicate SEM.

reduced the extent of melanin dispersion as compared with the crabs that received any of these drugs alone.

Rauwolscine significantly accelerated the rate of melanin dispersion (Fig. 6B). However, it did not affect significantly the HA-produced decrement in melanin dispersion when co-administered with HA.

6-OHDA was used as a pretreatment. This compound was injected into crabs with maximally concentrated melanin on a white background. One hour later these crabs were separated into two groups, one receiving HA and the other saline. In like manner, when the crabs were pretreated with the 6-OHDA, other crabs were pretreated with saline, and one hour later were divided into two groups, one that received HA and the other only saline. Immediately after receiving the second injection all of the crabs were transferred from the white background to a black one, and their melanophores were staged as usual for two hours. HA significantly inhibited melanin dispersion in the crabs pretreated with saline (Fig. 7). Likewise, the crabs that were pretreated with 6-OHDA and then received saline exhibited a significant reduction in their rate of melanin dispersion. On the other hand, HA did not significantly produce further inhibition of melanin dispersion in the crabs that had the 6-OHDA pretreatment.

DISCUSSION

The most logical hypothesis that can be deduced from the data presented above in light of the documented pharmacological actions of the drugs used herein in mammals is that HA H_1 and H_2 receptors are both present on NE neurons that control MDH release in the fiddler crab, *Uca pugilator*, and that administered HA

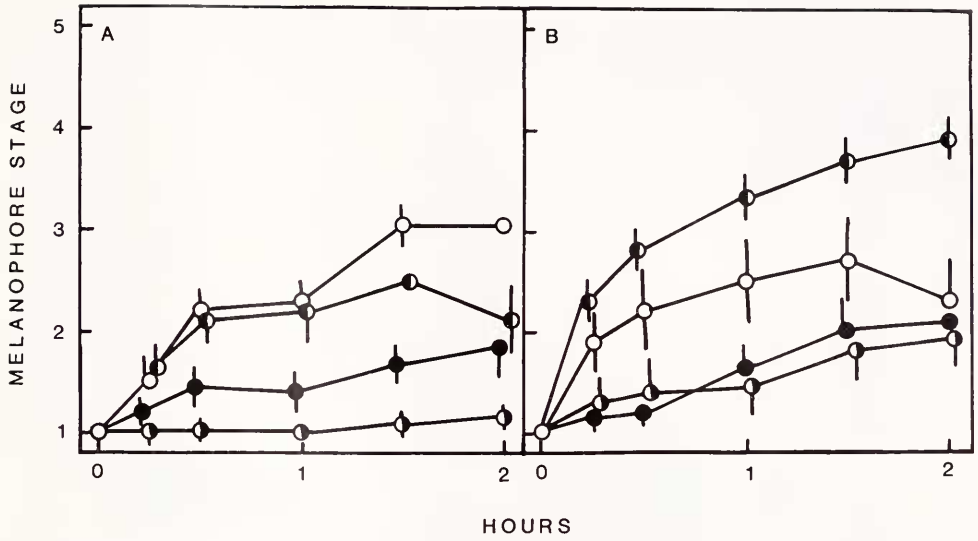

FIGURE 6. Relationships between melanophore stage and time for fiddler crabs, *Uca pugilator*, transferred from a white to a black background. (A): Solid circles, crabs that received histamine; circles half-filled on left, crabs that received bretylium; circles half-filled on right, crabs that received histamine plus bretylium; open circles, saline-injected controls. (B): Solid circles, crabs that received histamine; circles half-filled on left, crabs that received rauwolscine; circles half-filled on right, crabs that received histamine plus rauwolscine; open circles, saline-injected controls. Vertical bars indicate SEM.

inhibits MDH release by decreasing impulse-dependent noradrenergic neurotransmission through excitation of H_2 receptors. The effects of the drugs used herein are summarized in Table IV. The data presented in Figures 4B–7 also reinforce the hypothesis proposed earlier that NE triggers MDH release in this fiddler crab (Hanumante *et al.*, 1980). NE produces melanin dispersion in intact fiddler crabs but not in isolated legs. Consequently, the site of action of injected NE is considered to be the central nervous system. Furthermore, none of the histaminergic (Table III) or noradrenergic agents used in the present investigation affect melanin translocation directly (Hanumante and Fingerman, 1982b, c, and unpublished data), which is in keeping with their well proven effects on the nervous systems of other organisms and the noninnervated nature of fiddler crab chromatophores.

As stated earlier, at least in mammals HA receptors are classified into two types, called H_1 and H_2, depending upon their selective reactivity to specific histaminergic agonists and antagonists (Black *et al.*, 1972; Douglas, 1980; Schwartz *et al.*, 1980; Polanin and McNeill, 1981; Polanin *et al.*, 1981). For example, H_1 receptors are stimulated by 2-MeHA and 2-thiazolylethylamine and blocked by mepryramine and SA-97, whereas 4-MeHA and impromidine selectively stimulate H_2 receptors, and cimetidine and metiamide selectively antagonize H_2 receptors. Several recent reports reveal that in mammals HA can inhibit noradrenergic neurotransmission by an action on H_2 receptors located presynaptically on NE neurons (McGrath and Shepherd, 1976; Lokhandawala, 1978; Westfall, 1980; Vanhoutte *et al.*, 1981).

The absence of any melanin dispersion in the crabs administered the histaminergic agents and maintained throughout the experiment in white pans (and whose melanin was initally maximally concentrated) (Table I) is consistent with both the ability of a white background to foster melanin concentration and the pharma-

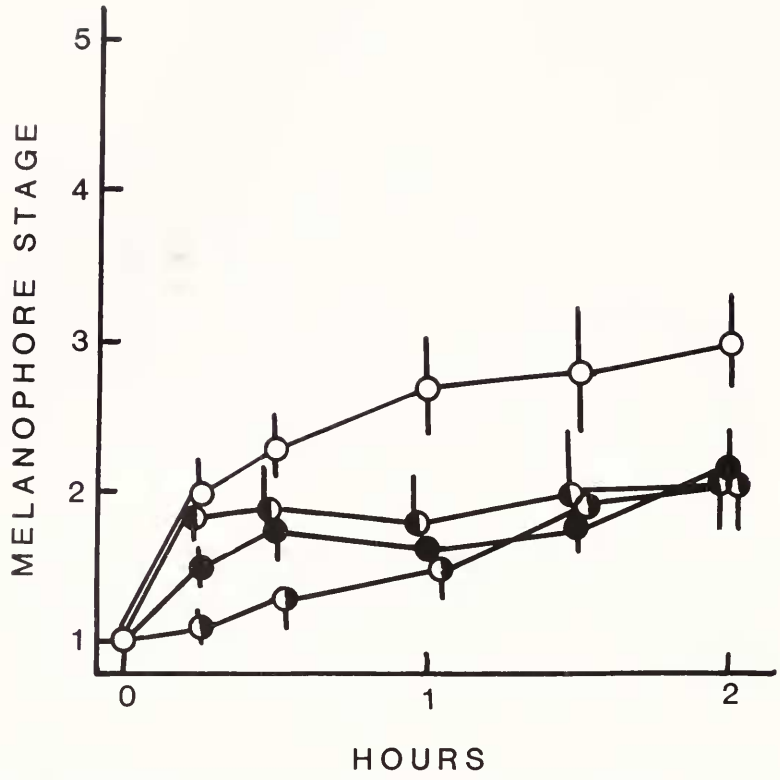

FIGURE 7. Relationships between melanophore stage and time for fiddler crabs, *Uca pugilator*, transferred from a white to a black background. Solid circles, crabs that received a 6-hydroxydopamine pretreatment followed by histamine; circles half-filled on left, crabs that received a pretreatment of 6-hydroxydopamine followed by saline; circles half-filled on right, crabs that received a pretreatment of saline followed by histamine; open circles, crabs that received two successive injections of saline alone (controls). Vertical bars indicate SEM.

cological actions of these drugs. The significant inhibition in the rate of centrifugal melanin migration produced by HA (an agonist of H_1 and H_2 receptors) and 4-MeHA (an agonist of H_2 receptors alone) in the crabs having maximally concentrated melanin initially and which underwent a background shift from white to black (Figs. 1, 4A, Table II) appears to be due to activation of H_2 receptors located on NE neurons. A black background, as stated above, fosters melanin dispersion, which will be effected by MDH. Consequently, on transferring the crabs from a white to a black background, neurogenic NE release would be initiated and/or increased which in turn would evoke MDH release by activating mainly $alpha_1$ adrenoceptors (Hanumante and Fingerman, 1981, 1982a). However, stimulation of H_2 receptors by HA and 4-MeHA would decrease stimulus-coupled NE secretion, and in turn less MDH would be released into the hemolymph. In mammals and the marine mollusk, *Aplysia californica*, HA is synthesized from L-histidine by L-histidine decarboxylase (Weinreich and Yu, 1977; Schwartz *et al.*, 1980). Presumably this enzyme required for the biosynthesis of HA is also present in the fiddler crab and the inhibitory effect of L-histidine on melanin dispersion (Table

TABLE IV

Summary of pharmacology and effects of the drugs used herein on melanin dispersion within melanophores of Uca pugilator *that underwent a background change from white to black.*

Drug	Pharmacology	Effect on melanin dispersion
Histamine	Stimulation of H_1 and H_2 receptors	−
2-Methyl histamine	Stimulation of H_1 receptors	+
4-Methyl histamine	Stimulation of H_2 receptors	−
SA-97, Pyrilamine	Blockade of H_1 receptors	None
Metiamide	Blockade of H_2 receptors	None
L-Histidine	Histamine precursor	−
Coryanthine	Blockade of α_1 adrenoceptors	−
B-HT 933	Stimulation of α_2 adrenoceptors	−
Rauwolscine	Blockade of α_2 adrenoceptors	+
Bretylium	Noradrenergic neuron blockade	−
6-Hydroxydopamine	Destruction of catecholamine neuroterminals	−

None of the drug significantly affected melanin translocation *in vitro*.
+ = Potentiation of melanin dispersion; − = inhibition of melanin dispersion.

II) in the crabs was due to the decarboxylation of this compound to HA which in turn stimulated H_2 receptors.

The end-organ responses produced by H_1 and H_2 receptors, when both are present on the same cell, are usually antagonistic to each other (Donoso and Banzan, 1980; Ganong, 1980; Gotow *et al.*, 1980; Geller, 1981). For example, stimulation of H_1 receptors increases plasma adrenocorticotropic hormone (ACTH) and corticoids whereas H_2 receptor activation has an inhibitory effect on the release of ACTH and consequently of corticoids (Ganong, 1980). In this context, H_1 receptors in *Uca pugilator* appear to be facilitatory, that is, their stimulation enhances stimulus-coupled NE secretion. In the crabs which underwent a background change from white to black and whose melanin was initially maximally concentrated, 2-MeHA an agonist of H_1 receptors, produced increased melanin dispersion (Table II, Fig. 3A), presumably by inducing increased NE secretion by stimulating H_1 receptors which in turn would produce a greater amount of MDH release into the hemolymph. However, when HA, which as stated above can stimulate both H_1 and H_2 receptors, was injected, the H_2 receptor-mediated inhibition on NE release apparently predominated because HA produced decreased melanin dispersion.

In the crabs maintained throughout the experiment on a black background and whose melanin was initially maximally dispersed, impulse-mediated NE release (and in turn MDH secretion) would be occurring at a greater rate than in crabs on a white background. Consequently, the H_1 receptor agonist 2-MeHA would facilitate stimulus-coupled NE release whereas H_2 receptor agonists such as 4-MeHA would inhibit stimulus-coupled NE release, thus accounting for the results presented in Table I for these substances, 2-MeHA fostering melanin dispersion in crabs on a black background and 4-MeHA reducing melanin dispersion.

In crabs having maximally dispersed melanin and which are transferred from a black to a white background, presumably the impulses calling for NE-mediated MDH release are terminated or reduced. Consequently, HA and its precursor L-histidine would be expected not to diminish NE-coupled MDH secretion in crabs transferred from a black to a white background as in crabs transferred from a

white to a black background, which was the case (Table II). The small but significant decrease in the rate of melanin concentration produced by 2-MeHA and the increase produced by 4-MeHA were presumably due respectively to rapid increment (through H_1 receptors) or decrement (through H_2 receptors) in impulse-mediated NE release just before the appropriate melanin-concentrating events were initiated following the background change from black to white (Table II).

The inability (Fig. 2A) of the H_1 receptor blockers pyrilamine and SA-97 and the H_2 receptor blocker metiamide to affect melanin translocation when administered alone in a concentration (20 μg/dose) which significantly affected administered H_1 and H_2 receptor agonists (Figs. 2–4A) is not a unique phenomenon. In dogs H_1 and H_2 receptor antagonists did not alter the response to sympathetic nerve stimulation, even though injected HA produces an inhibitory effect on such noradrenergic neurotransmission by an action on H_2 receptors (Lokhandawala, 1978). Similarly, in dogs a dose of mepyramine (pyrilamine) that blocked the stimulatory effect of intraventricularly injected HA failed by itself to produce a significant decrease in ACTH and plasma corticoids (Rudolph *et al.*, 1979).

The H_1 receptor blockers, when co-administered with HA, were unable to abolish HA-induced melanin inhibition (Fig. 2B). This indicates that receptors other than the H_1 type are involved in the HA-mediated melanin inhibition. The data presented in Figure 3A substantiate this interpretation, revealing not only that H_1 receptor stimulation by 2-MeHA produced increased melanin dispersion but also that H_1 receptor blockers significantly prevented the usual increase in melanin dispersion in response to 2-MeHA administration. Metiamide significantly blocked the actions of HA and 4-MeHA (Figs. 3B and 4A), thereby indicating that H_2 receptors mediate the HA inhibition of melanin dispersion in fiddler crabs transferred from a white to a black background.

The data in Figures 4A–6A clearly demonstrate that administered HA is not interacting with the postsynaptic alpha$_1$ adrenoceptors, through whose activation NE mainly elicits MDH release (Hanumante and Fingerman, 1981, 1982a), or with the presynaptic alpha$_2$ adrenoceptors which regulate local NE release (Fingerman *et al.*, 1981; Hanumante *et al.*, 1981; Hanumante and Fingerman, 1981, 1982c). Coryanthine, the alpha$_1$ adrenoceptor blocker, antagonizes at least partially the MDH-releasing action of NE, the secretion of which would be initiated or augmented because of a background change from white to black. Therefore, in control crabs the melanin would disperse at a greater rate than in coryanthine-administered crabs, as seen in Figures 4B and 5A. In the crabs which received the combination of HA and coryanthine, not only would coryanthine, but, as discussed above, HA would also ultimately diminish MDH secretion. In the case of the crabs co-administered 4-MeHA and coryanthine (Fig. 5A), the combination of alpha$_1$ adrenoceptor-blocking and H_2 receptor-stimulating drugs presumably produced such a strong reduction in MDH release that the extent of melanin dispersion was significantly less than in the crabs injected with 4-MeHA alone.

The pharmacological effects (Fig. 5B) of B-HT 933, an alpha$_2$ adrenoceptor agonist, are also consistent with the earlier reports from this laboratory (Hanumante and Fingerman, 1981, 1982c). Presynaptic alpha$_2$ adrenoceptors have been implicated in a local negative feedback mechanism regulating release of NE, which, as stated above, triggers MDH secretion in *Uca pugilator*. Agonists of alpha$_2$ adrenoceptors diminish and antagonists of these adrenoceptors enhance impulse-dependent NE release (Langer, 1977; Starke, 1977; Vanhoutte *et al.*, 1981). The combined treatment of B-HT 933 and HA (Fig. 5B) produced further significant

reduction in the extent of melanin dispersion over that induced by either drug alone. Furthermore, rauwolscine significantly offset the B-HT 933-induced decrement in the rate of melanin dispersion (Hanumante and Fingerman, 1982c) but not the HA-induced decrement (Fig. 6B). These observations unambiguously reveal that even though B-HT 933 and HA evoke similar end-organ responses in the fiddler crab, namely inhibition of melanin dispersion, the mechanisms of action of these agents are quite dissimilar; the former stimulating alpha$_2$ adrenoceptors and the latter exerting its effect by stimulating H_2 receptors, both receptors being situated presynaptically on NE neurons.

Bretylium, the noradrenergic neuron blocker which decreases action potential-linked NE release, slows the rate of centrifugal melanin migration in fiddler crabs having maximally concentrated melanin initially and which undergo a background change from white to black (Hanumante and Fingerman, 1982b). The fact that HA when co-administered with bretylium (Fig. 6A) further significantly decreased the rate of centrifugal melanin migration shows that these two drugs can act synergistically to inhibit melanin dispersion even though their mechanisms of action are different. Furthermore, HA does not enhance neuronal uptake of NE to ultimately reduce the rate of melanin dispersion nor does it affect other neurotransmitters known to be involved in triggering the release of other chromatophorotropic hormones in the fiddler crab (unpublished data). Therefore, we are left with the hypothesis mentioned earlier that H_2 receptor activation is responsible for the HA-mediated inhibition of melanin dispersion in crabs having maximally concentrated melanin initially and undergoing a background change from white to black.

6-OHDA inhibits the rate of melanin dispersion in fiddler crabs, *Uca pugilator*, having maximally aggregated melanin initially and transferred from a white background to a black one (Hanumante and Fingerman, 1982b). The fact that of the possible neurotransmitters tested at 8 μg/dose (including octopamine, NE, dopamine, and epinephrine) by Fingerman *et al.* (1981) only NE induced melanin dispersion in the fiddler crab strongly suggests that the inhibition of melanin dispersion produced by 6-OHDA was due to its destructive action on noradrenergic terminals. This, together with the failure of HA to further reduce significantly the rate of centrifugal melanin migration in crabs pretreated with 6-OHDA (Fig. 7), clearly indicates that the integrity of noradrenergic nerve terminals is essential for HA to exert its effect. That is, the H_2 receptors through whose activation HA produced an inhibition of melanin dispersion must be situated presynaptically on NE neurons.

It is interesting from the point of evolution and comparative pharmacology that in the marine mollusks *Aplysia californica* (Carpenter and Gaubatz, 1975; Gruol and Weinreich, 1979) and *Ochidium verruculatum* (Gotow *et al.*, 1980) two types of HA receptors corresponding to but not exactly the same as the mammalian H_1 and H_2 receptors, have been reported. For example, two temporally and ionically dissimilar hyperpolarizing responses were produced in *Aplysia californica* when HA was applied to neurons in the cerebral ganglion. The use of H_1 and H_2 receptor agonists and antagonists revealed that two distinct HA receptor types mediated these hyperpolarizing responses. However, the responses of these neurons to H_1 and H_2 reagents often differed from what has been found with mammals; the drugs were often non-selective or ineffective in *Aplysia*. In view of this difference between molluscan and mammalian histaminergic receptors and the need for due caution in interpretation of the data on invertebrate neuronal mechanisms obtained using agents specific for mammalian nervous systems, it would be worthwhile to com-

pletely characterize the HA receptors in *Uca pugilator* (*i.e.,* pharmacologically, biochemically, and physiologically) in order to determine their similarity (or dissimilarity, if any) to those of the mammals and other invertebrates.

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